Title: Molecular Genetics of the R Complex of Maize

Summary
A molecular genetic characterization of the maize R-r complex of maize was completed during the period of support. The complex was shown to consist of two main regions: the P region, containing the r-p gene which controlled pigmentation of plant parts, and the S subcomplex, containing two rI-s genes in head-to-head orientation and a non-functional component termed rI-q. By examining the DNA sequences at the junction of the rI genes, the complex was shown to be derived by a series of abortive transposition events. The transposable element involved in the gene duplication and rearrangements was characterized and called doppia. Meiotic instability of the R-r complex was also characterized. Loss of P or S function was associated with several structural changes including intrachromosomal recombination and excision of a novel transposable element that appears to show instability only during meiosis.

Specific Research Accomplishments and Publications:


Previous studies have suggested that the R locus of maize is responsible for determining the temporal and spatial pattern of anthocyanin pigmentation in the plant. In this report we demonstrate that three members of the R gene family, P, S, and Lc, encode homologous transcripts 2.5 kilobases in length. The structure of one R gene, Lc, was determined by sequencing cDNA and genomic clones. The putative Lc protein, deduced from the cDNA sequence, is composed of 610 amino acids and has homology to the helix-loop-helix DNA-binding/dimerization motif found in the L-myc gene product and other regulatory proteins. It also contains a large acidic domain that may be involved in transcriptional activation. Consistent with its proposed role as a transcriptional activator is our finding that a functional R gene is required for the accumulation of transcripts of at least two genes in the anthocyanin biosynthetic pathway. We discuss the possibility that the diverse patterns of anthocyanin pigmentation conditioned by different R genes reflect differences in the R gene promoters rather than their gene products.


The R complex of Zea mays encodes a tissue-specific transcriptional activator of the anthocyanin pigment biosynthetic pathway. Certain R alleles comprise two genetically
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distinct components that confer the plant (P) and seed (S) aspects of the pigmentation pattern. These alleles are meiotically unstable, losing (P) or (S) function, often accompanied by exchange of flanking markers. We show that the (P) component consists of a single gene within the R-r complex, whereas the (S) component is part of a more complex arrangement of multiple R genes or gene subfragments. A third, cryptic region of the complex, termed (Q), consists of a truncated R sequence. The analysis of R-r crossover derivative alleles shows they arise from unequal exchange between the (P) gene and one of several distinct regions of the R-r complex. Restriction site polymorphisms were used to show that most of these unequal exchanges are intragenic. The frequency of displaced intragenic recombination is comparable to previous estimates for intragenic recombination in maize involving genes that are not duplicated. These exchange events have been used to determine the arrangement of components within the complex and their orientation in the chromosome. We also show that localized rearrangements in the (P) or (S) components are responsible for noncrossover derivative alleles. The organization of R-r has implications for these noncrossover derivatives and models for their origin are discussed.


The Sn locus of maize is functionally similar to the R and B loci, in that Sn differentially controls the tissue-specific deposition of anthocyanin pigments in certain seedling and plant cells. We show that Sn shows molecular similarity to the R gene and have used R DNA probes to characterize several Sn alleles. Northern analysis demonstrates that all Sn alleles encode a 2.5 kb transcript, which is expressed in a tissue-specific fashion consistent with the distribution of anthocyanins. Expression of the Sn gene is light-regulated. However, the Sn: bol3 allele allows Sn mRNA transcription to occur in the dark, leading to pigmentation in dark-grown seedlings and cob integuments. We report the isolation of genomic and cDNA clones of the light-independent Sn: bol3 allele. Using Sn cDNA as a probe, the spatial and temporal expression of Sn has been examined. The cell-specific localization of Sn mRNA has been confirmed by in situ hybridization using labelled antisense RNA probes. According to its proposed regulatory role, expression of Sn precedes and, in turn, causes a coordinate and tissue-specific accumulation of mRNA of structural genes for pigment synthesis and deposition, such as A1 and C2. The functional and structural relationship between R, B, Lc and Sn is discussed in terms of an evolutionary derivation from a single ancestral gene which gave rise this diverse gene family by successive duplication events.

R-r controls the production of anthocyanin pigment in plant parts and the aleurone layer of seeds through the production of a family of related transcriptional activating proteins of the helix-loop-helix type. The R-r complex comprises a series of repeated, homologous components arranged in both direct and inverted orientations. These include the P component, a simple R gene that confers pigmentation of plant parts, and the S subcomplex that consists of a truncated inactive R gene called q, and two functional R genes, S1 and S2, that pigment the aleurone. The S genes are arranged in an unusual inverted head-to-head orientation. The identity of each functional component was confirmed by microprojectile bombardment of intact maize tissues with cloned genomic DNA and by analysis of in vivo mRNA populations. Sequence analysis suggests that the S subcomplex was derived through the rearrangement of a simple P-like progenitor element. At the rearrangement breakpoints, features typical of the CACTA family of transposable elements were found. The location and arrangement of these CACTA element sequences implies that this element may have mediated the chromosomal rearrangements that led to the formation of the R-r complex. The unusual structure of R-r explains much of the meiotic instability of the complex.


The r locus of maize regulates anthocyanin synthesis in various tissues of maize through the production of helix-loop-helix DNA binding proteins capable of inducing expression of structural genes in the anthocyanin biosynthetic pathway. The complex r variant, R-r: standard (R.r), undergoes frequent mutation through a variety of mechanisms including displaced synapsis and crossing over, and intrachromosomal recombination. Here we report a new mechanism for mutation at the R-r complex: insertion of a novel family of transposable elements. Because the elements were first identified in the R-p gene of the R-r complex, they have been named P instability Factor (PIF). Two different PIF elements were cloned and found to have identical sequences at their termini but divergent internal sequences. In addition, the PIF elements showed a marked specificity of insertion sites. Six out of seven PIF-containing derivatives examined had an element inserted at an identical location. Two different members of the PIF element family were identified at this position. The seventh PIF-containing derivative examined had the element inserted at a distinct position within r. Even at this location, however, the element inserted into a conserved target sequence. The timing of PIF excision is unusual. Germinal excision rates can range up to several percent of progeny. Yet somatic sectors are rare, even in lines exhibiting high germinal reversion rates.